Zeta potential examination of β-carotene encapsulated in starch-chitosan/tripoly-phosphate microparticles

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Zeta Potential Examination of β-Carotene Encapsulated in Starch-Chitosan/Tripoly-phosphate Microparticles

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Abstract. This study examined zeta potential of some encapsulation products of β-carotene in the starch-chitosan/TPP matrices. the effects of weight ratio of starch to chitosan, β-carotene loading, and tripolyphosphate (TPP) addition level on zeta potential of the microparticle products were determined. The native and hydrolyzed starches as well as low molecular weight chitosan were used in the preparation of microparticles. The synthesis of microparticle was carried out by dropwise addition of ethanolic dispersion of β-carotene into the starch-chitosan/TPP aqueous mixture. The results showed that zeta potentials of all synthesized microparticles are between 5.8-13.2 mV with a negative charge, except for microparticle with the weight ratio of starch to chitosan of 4:16. The increase in β-carotene addition level from 10 to 20 mg did not change zeta potential charge. From the variation of TPP addition in the range of 400-600 mg, it was found that the highest colloidal stability was shown by TPP addition of 600 mg for the composition of weight ratio of starch to chitosan of 10:10 and β-carotene addition of 10 mg. These results also confirm that the encapsulation products exhibit colloidal instability in water.

INTRODUCTION

The development of nanoscale and microparticles in various fields has continuously increased because of their various unique properties over the same particle in a larger size. In the field of drug delivery, drugs are encapsulated in a polymer matrices to form nano/micro-sized particles. The advantages of this system are that it can regulate the release of drugs in the desired target system, increase storage stability, reduce drug side effects, increase absorption, and several other purposes[1]. The increase in emulsion stability and absorption can be monitored through a nano/micropraticle characteristic, namely zeta potential[2].

The zeta potential of a particle is an electric potential at the boundary of the double layer on the surface of the particle[3]. The surface charge of microparticles varies depending on the composition of the microparticles. A positive or negative value of the zeta potential indicates that the dispersed particles in the suspension that we measure have a positive or negative surface charge according to the zeta potential value. Nano/microparticles with positive and negative charges have advantages and disadvantages. Therefore, the surface charge density of nano/micro particles must be optimized so that a minimal level of toxicity is achieved, but it still has effective capability in drug delivery into cells. Both positive and negative charges are known to increase the rate of drug delivery to cells via adsorptive endocytosis. The positively charged nano/microparticles have several advantages in the drug delivery system because the cell surface is negatively charged so that the drug is more easily absorbed, but the cationic charge is claimed to increase cytotoxicity. The positive charge has a destabilizing and destructive effect on the membrane resulting from the interaction of positive nano/microparticle charge and negative membrane charge. On the other hand, negatively charged particles do not easily accumulate in the lymph nodes compared to positively charged particles, but they have the disadvantage of being cleaned more slowly from the bloodstream[2].

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The zeta potential, besides showing the surface charge of the particles, is also an indicator of the stability of the suspension. A larger charge on the surface of the nano/microparticles will prevent the aggregation of particles in the solution because of the greater repulsion between the particles[4]. The absolute value of the zeta potential can be from zero to one hundred millivolts (mV)[5]. The generally accepted threshold for the zeta potential of a stable aqueous suspension is about 30 mV[6].

In this study, we have synthesized β-carotene micropraticles encapsulated in the matrix of starch-chitosan/TPP and measured their zeta potential as well as discussed the results from the point of view of their compositions. Starch and chitosan are natural polymers. Structure of starch consists of amylose and amylopectin[7] and the monomer of starch is glucose[7], while the monomers of chitosan are D-glucosamine and N-acetylglucosamine[8]. The chemical structure of materials used in this study are shown in Figure 1.

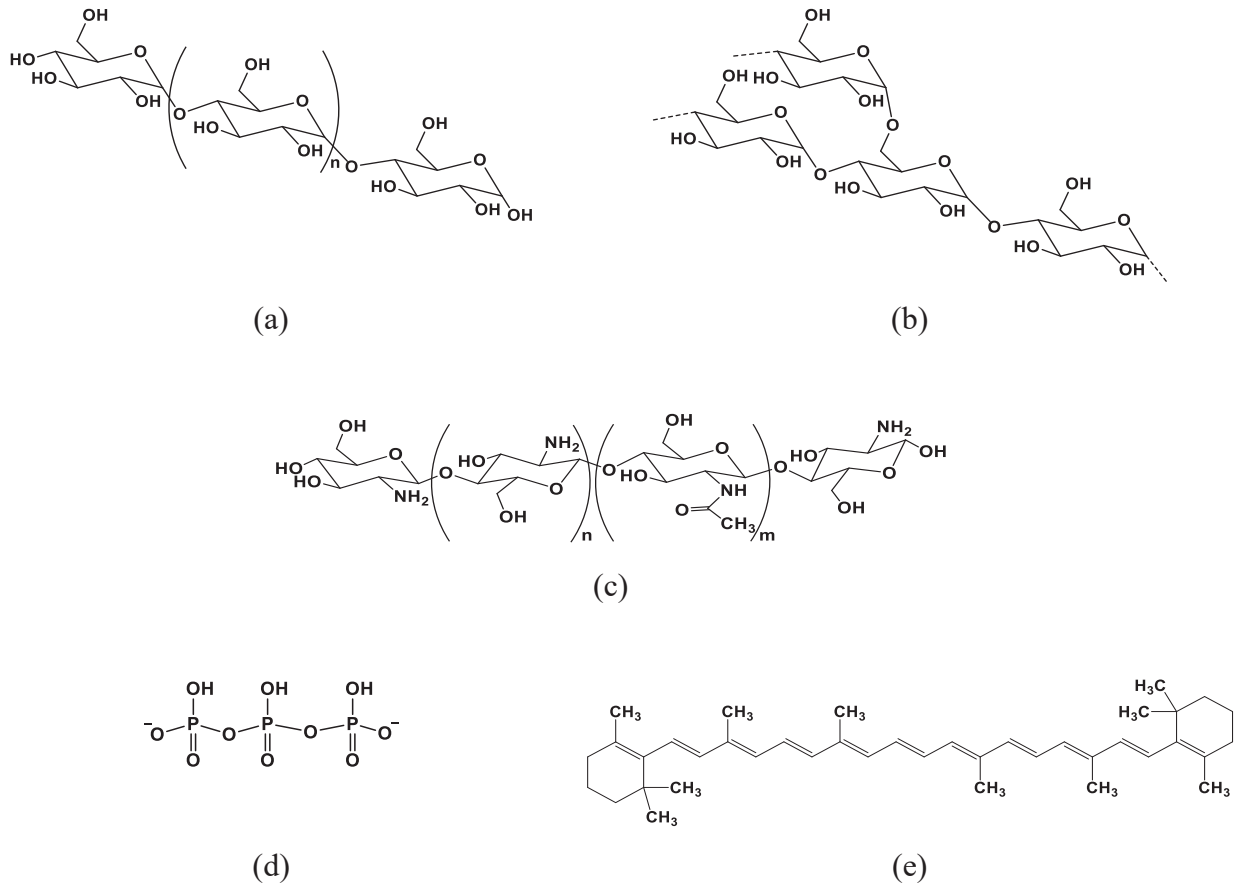

FIGURE 1. Chemical structure of (a) amylose, (b) amylopectin, (c) chitosan, (d) TPP, and (e) β-carotene

MATERIALS AND METHODS

The synthesized method of β-carotene micropraticles encapsulated in the matrix of starch-chitosan/TPP used in this study wis in accordance with the method previously reported in our study[9] as described below.

Materials

β-Carotene, corn starch, chitosan and TPP from Sigma Aldrich; glacial acetic acid, HCl, and ethanol from Merck, and demineralized water.

Methods

β-Carotene Microencapsulation in The Starch-chitosan/TPP Matrix

The synthesis process of β-carotene encapsulated in the starch-chitosan/TPP matrix was carried out as in our previous study[9] which based on the steps of Kim and Huber's[10]. The starches used in this study are native starch and acid hydrolyzed starch (that was made by reacting native starch with 0.15 M HCl for 8 hours at 50 °C[11]). First, we added 20 mL of TPP solution to the 1% starch dispersion according to the volume given in Table 1, and stirred it using magnetic stirer. Then we poured chitosan solution into the mixture of starch-TPP. The starch-in-water dispersion was prepared by weighing the starch according to the weight required to form a starch dispersion with a concentration of 1%. TPP solutions were prepared by dissolving a certain amount of TPP in water until the desired TPP concentration was obtained. The chitosan solution was made by dissolving chitosan in a 1% acetic acid solution for 1 night using a magnetic stirrer.

After the matrix mixture was prepared, it was heated for 10 minutes at 90 °C in a water bath equipped with a magnetic stirrer. The β-carotene dispersion was added to the matrix mixture in 2 steps dropwise each with a volume of 100 mL. The first stage is carried out at 90 °C, the second stage at room temperature. Next, the mixture was left to stand at room temperature, stored in a refrigerator and subjected to centrifuge at 7000 × g for 10 min. The precipitate obtained was dried in a freeze-dryer for 13 hours, crushed, and sieved through a 200 mesh sieve.

TABLE 1. Composition of matrices

Sample code*	Weight ratio of S/C	β-carotene conc. (mg/200mL)	TPP conc. (%)	Volume of 1% acid hydrolyzed starch dispersion (mL)	Volume of 1% chitosan solution (mL)	Volume of TPP solution (mL)
SC 4:16	4:16	10	3	40	160	20
SC 10:10	10:10	10	3	100	100	20
SC 16:4	16:4	10	3	160	40	20
B10	10:10	10	3	100	100	20
B20	10:10	20	3	100	100	20
T400	10:10	10	2	100	100	20
T600	10:10	10	3	100	100	20
T800	10:10	10	4	100	100	20

*sample codes are designated as follows: the SC stands for starch-chitosan, the numbers following the SC letters were the weight ratio of starch to chitosan, the B letter represents β-carotene, the number after the B letter is the addition level of β-carotene, the T letter stands for TPP, and the number at the end of the sample code denotes the TPP addition level.

Analysis of Zeta Potential

Zeta potensial of the microparticles was determined by Horiba SZ-100 (Horiba, Ltd., Japan). The product of microencapsulation was added with demineralized water and then it was homogenized for 1 minute and analyzed using zetasizer equipped in DLS (Dynamic Light Scattering) instrument.

RESULTS AND DISCUSSION

In this study, zeta potential has been measured for several samples of β-carotene microparticles encapsulated in the native starch-chitosan/TPP matrix and β-carotene encapsulated in the hydrolyzed starch-chitosan/TPP matrix. The results of zeta potential measurement in this experiment reveal that the zeta potential values of the samples are between 5.8-13.2 mV (see Figure 2, 3, and 4).

Effect of Starch to Chitosan Weight Ratio

In Figure 2, we can see that the zeta potential is positive at the starch/chitosan weight ratio of 4:16 for both β-carotene encapsulated in native starch-chitosan/TPP and hydrolyzed starch-chitosan/TPP microparticles. This is because at the starch/chitosan weight ratio of 4:16 there are still approximately 0.00131 moles of $–NH_3^+$ groups remain in the matrices because it is not cross-linked by TPP and also not hydrogen-bound to starch. At the starch/chitosan weight ratio of 4:16, we understand from Table 2 that there are 0.00852 moles of chitosan (0.00679 mol D-glucosamine and 0.00174 mol N-acetyl glucosamine); 0.00222 moles of starch and 0.00163 moles of TPP (Table 2).

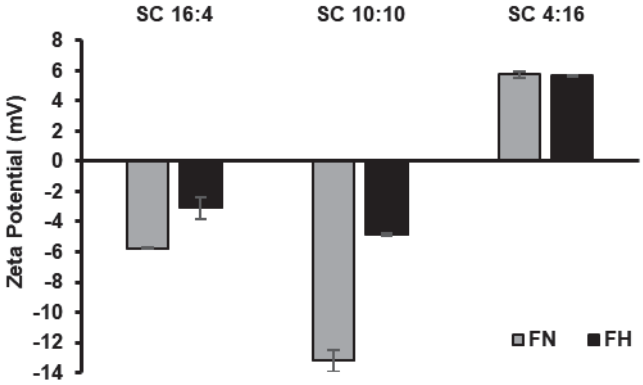

FIGURE 2. Zeta potential of encapsulated products using native starch-chitosan/TPP matrix and hydrolyzed-chitosan/TPP starch at different weight ratio of starch to chitosan (FA = encapsulated products using native starch-chitosan/TPP matrix, FH = encapsulated products using hydrolyzed-chitosan/TPP starch matrix, SC = weight ratio of starch/chitosan)

TABLE 2. Moles of carotene, starch, chitosan, and TPP

Fomulation Code	weight of starch (g)	weight of chitosan (g)	mole of starch as glucose (mol)	mole of D-glucosamine (mol)	mole of N-acetylglucosamine (mol)	mole of chitosan*) (mol)	mole of TPP (mol)	Excess in the mole of TPP*) (mol)
SC 4:16	0.4	1.6	0.00222	0.00679	0.00167	0.00852	0.00163	-0.52
SC 10:10	1	1	0.00555	0.00424	0.00104	0.00533	0.00163	-0.23
SC 16:4	1.6	0.4	0.00888	0.00170	0.00042	0.00213	0.00163	0.92
B5	1	1	0.00555	0.00424	0.00104	0.00533	0.00163	-0.23
B20	1	1	0.00555	0.00424	0.00104	0.00533	0.00163	-0.23
T400	1	1	0.00555	0.00424	0.00104	0.00533	0.00109	-0.49
T600	1	1	0.00555	0.00424	0.00104	0.00533	0.00163	-0.23
T800	1	1	0.00555	0.00424	0.00104	0.00533	0.00217	0.03

*sample codes were designated as follows: the SC letters abbreviate starch-chitosan, the numbers following the SC letters were the weight ratio of starch to chitosan, the B letter represented β-carotene, the number after the B letter was the addition level of β-carotene, the T letter was stand for TPP, and the number at the end of the sample code denoted the TPP addition level.

The zeta potential of microparticles at the starch/chitosan weight ratio of 10:10 and 16: 4 are all negative. At the weight ratio of starch/chitosan 10:10 there were 0.00533 moles of chitosan (0.00424 mol D-glucosamine and 0.00108 mol N-acetyl glucosamine); 0.00555 moles of starch and 0.00163 moles of TPP. Although there is a residue of 0.00098 mol $–NH_3^+$, this group can be hydrogen-bonded to the –OH group in starch. The dispersion of starch in water provides a negative zeta potential[12]. The excess of 0.00457 mol of free starch resulted in a negative zeta

potential at the weight ratio of starch/chitosan 10:10. This indicates that the zeta potential of starch is more dominant than that of chitosan in this weight ratio level. At the 16:4 starch/chitosan weight ratio there are 0.00213 moles of chitosan (0.00170 mol D-glucosamine and 0.00043 mol N-acetyl glucosamine); 0.00888 moles of starch and 0.00163 mol of TPP, so that there is still excess of 0.00156 mol of TPP that does not react with $–NH_3^+$. Therfore, we understand that the remaining TPP in the matrices gives negative zeta potential of the starch/chitosan weight ratio 16: 4.

Effect of β-Carotene Addition Level

The charge type of zeta potential does not change, i.e. remained negative, by increasing addition of the weight of β-carotene in the β-carotene microparticles encapsulated in native starch-chitosan/TPP (Figure 3). This is because the amount of β-carotene addition to the microparticles is relatively small so that it does not able to affect the surface charge of the matrices.

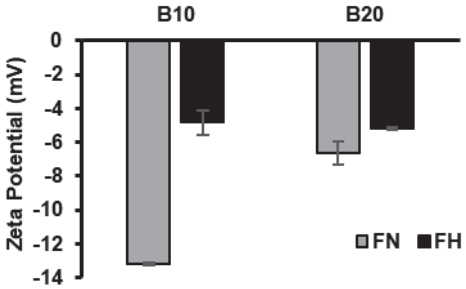

FIGURE 3. Zeta potential of encapsulated products using native starch-chitosan/TPP matrix and hydrolyzed-chitosan/TPP starch in different β-carotene addition level (FA = encapsulated products using native starch-chitosan/TPP matrix, FH = encapsulated products using hydrolyzed-chitosan/TPP starch matrix, B = β-carotene addition level (mg))

Effect of TPP Addition Level

The zeta potential of microparticles remained negative at TPP level in the range of 400 and 800 mg (Figure 4). At TPP addition of 400 mg, the product of encapsulation contained 0.00533 moles of chitosan (0.00424 mol D-glucosamine and 0.00108 mol N-acetyl glucosamine); 0.00555 moles of starch and 0.00109 moles of TPP. At this level, actually there is still an excess of 0.00206 moles of the $–NH_3^+$ group. However, this group undergoes hydrogen bonding with starch and leaves 0.00349 moles excess of starch, therefore the zeta potential of the microparticle becomes negatively charged.

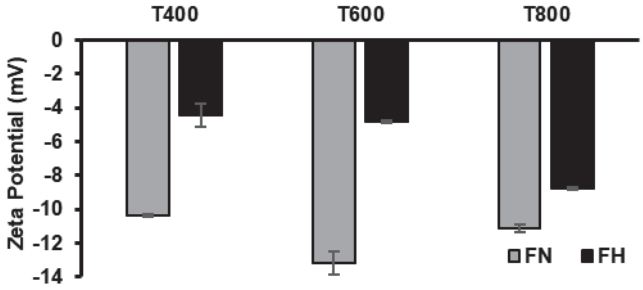

FIGURE 4. Zeta potential of encapsulated products using native starch-chitosan/TPP matrix and hydrolyzed-chitosan/TPP starch in different TPP addition level (FA = encapsulated products using native starch-chitosan/TPP matrix, FH = encapsulated products using hydrolyzed-chitosan/TPP starch matrix, T = TPP addition level (mg))

The mole ratio in the T600 sample is the same as that of the SC 10:10 sample because it is indeed the same sample. For the addition of 800 mg of TPP weight, there are 0.00533 moles of chitosan (0.00424 mol D-

glucosamine and 0.00108 mol N-acetyl glucosamine); 0.00555 moles of starch and 0.00217 moles of TPP. At this TPP addition level, microparticle still has an excess of 0.0001 mol TPP, this excess gives the zeta potential to have negative value.

CONCLUSIONS

The results show that zeta potential of β-carotene microparticles encapsulated in the native starch-chitosan/TPP matrix and β-carotene encapsulated in the hydrolyzed starch-chitosan/TPP matrix is in the range of between 5.8-13.2 mV. This results indicates the the encapsulated products are colloidal instability in water. Almost all of the microparticles have negative zeta potential, except those for microparticles with a weight ratio of starch to chitosan 4:16. The addition of β-carotene levels from 10 to 20 mg does not change the zeta potential charge. At an increase in TPP addition level between 400-800 mg, the highest stability is found in products with a TPP addition level of 600 mg (weight ratio of starch/chitosan 10:10 and addition of β-carotene 10 mg). Regardless of the type of particle charge, the encapsulated β-carotene product in the native starch-chitosan TPP matrix had a lower zeta potential than the β-carotene product encapsulated in the chitosan-hydrolyzed starch/TPP matrix.

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